"STUDY OF MYCOTOXIC EFFECT AND CONTROL *ASPERGILLUS NIGER* INFECTION OF LEGUMINOSAE (FABACEAE) WITH SPECIAL REFERENCE TO *ARACHIS HYPOGAEA*(GROUNDNUT)"

"एरेकिस हाइपोगिया के विशेष संदर्भ में लेग्यूमिनोसी (फैबेसी) कुल पर एस्पर्जिलस नाइजर संक्रमण के विषैले प्रभाव एवं नियंत्रण का अध्ययन"

A

SYNOPSIS

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By
Ms. Suman Lora
Under Supervision of

Dr. Tahira Begum
Lecture
Department Of Botany
S.P.C. Govt. College Ajmer

INTRODUCTION

The fungi are major disease causing agents on plants and can lose up to 90% agricultural yield. Various systemic fungicides have been used to control the plant diseases. But due to indiscriminate use of synthetic fungicides, various important pathogens have been developed resistance to many of the currently available fungicides (Gangawane, 1990).

Sometimes the fungicide adversely affect on the non target organisms. Hence it is necessary to search new antifungal compounds as an alternatives, safe, eco-friendly, cheap to synthetic fungicides from plants, since they produce different secondary metabolites which perform defensive role in plants and protect the plants from their invaders. Plant extracts and essential oils has been investigated throughout the world for their antifungal activity against wide range of fungi (Ezzat, 2001; Abd-El-Khair and Hafez, 2006; Gupta *et al.* 2008).(AE)

The most important method of protecting the plants against the fungal attack is the use of fungicides. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently effect the humans through the food chain. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some eco-friendly measures for the management of diseases.

2. Objectives

(i) Survey, Collection, Isolation, Purification and Pathogenicity of the fungus associated with disease.

(ii) To study the Cultural, Morphological and Pathogenic Variability of the fungus.

(iii) Management of Collar Rot Pathogen through various Chemicals and Bio-agents.

(iv) Screening of available Varities against Collar Rot.

3. Importance of the Proposed Investigation:

Groundnut is mainly grown as oil seed *kharif* crop in India. It is also known as peanut, earthnut, monkeynut, goobernut, panda and marillnut. De condole (1886) stated its origin to be in Brazil (South America). The genus *Arachis* comes under the family Fabaceae. The crop is principally cultivated as an oilseed but considerable quantities are used directly for human consumption like other pulses. The kernels are widely acknowledged as a rich and cheap source of vegetable protein. The chemical composition of the kernels, indicating their value as food is protein

(25.33%), carbohydrate (0.20%), fat (40.50%), fiber (3.4%) and ash (1.9%). However, as source of oil, the groundnut finds its largest use thus deriving the reputation as "King of oil" seed crops (Reddy, 1976).

The groundnut is presently grown on a commercial scale in about 82 different countries in the world in India. The total coverage area under this crop is about 5.5 million hectare and production 9.5 million tonnes and average productivity 1723 kg/hectare (Anonymous, 2013-14, a). Major growing states of India are Gujarat, Andhra Pradesh, Rajasthan and Tamilnadu. Among the states, Gujarat stand first in its production while Andhra Pradesh in area. Rajasthan stands seventh in terms of area and production both. The cultivation of groundnut is well adapted to the conditions prevailing in Rajasthan and is cultivated in about 0.46 million hectares with annual production 0.90 million tonnes and productivity 1950 kg/ hectare (Anonymous, 2013-14, b).

The major groundnut growing districts in Rajasthan are Jaipur, Bikaner, Sikar, Churu, Dausa, Udaipur and Nagaur

Among the districts, Bikaner stand first in its area and production followed by Jaipur. A major limiting factor in profitable cultivation of this crop in Rajasthan is the attack of several diseases mainly caused by fungi, which takes heavy loss of the crop at all the stages of growth right from sowing to harvest and storage.

Like all other crops, groundnut suffers from various diseases caused by fungi and other microorganisms. Important diseases incited by fungi are collar rot (*Aspergillus niger*), early leaf spot (*Cercospora arachidicola*), late leaf spot (*Phaeoisariopsis personata*), rust (*Puccinia arachidis*), root rot (*Macrophomina phaseolina*). The diseases, which affect the foliage cause extensive damage to tissues, interfere the photosynthetic process and cause severe losses in yield.

Amongst these diseases, collar rot of groundnut caused by *Aspergillus niger* is one of important seed and soil borne disease. This disease was first reported by Jochem (1926). However, Jain and Nema (1952) first reported the *Aspergillus* blight of groundnut caused by *A. niger* in India. It

is an important disease in the major groundnut growing areas of the states. The maximum disease incidence (25-50 %) was recorded in Rajasthan (Bakhetia, 1983).

The disease is prevalent in almost all groundnut growing states of India *viz*, Punjab, Gujarat, Andhra Pradesh, Tamilnadu, Uttar Pradesh, Maharastra, Rajasthan, Karnataka and Orissa. The disease causes more damage in sandy loam and medium black soils. The most of the groundnut cultivars are susceptible to this disease. It is emerging as a major and wide spread problem in Rajasthan. The pathogen seems to have adaptability to higher temperature and the disease occurs during July-Sept, which is particularly severe at pre and post emergence stages causing considerably losses to the yield.

A review of literature revealed that a very limited information is available on pathogen variability and management of this disease. The information are needed for proper developing/deploying effective disease management strategy. Considering the increasing importance of collar rot

of groundnut, it is proposed to undertake the investigations on above aspects.

4. REVIEW OF LITERATURE

4.1 Survey, Collection, Isolation, Purification and Pathogenicity

Singh *el al. (*2006) conducted survey during 2003-04 to investigate the incidence of lentil collar rot *(Sclerotium rolfsii)* in various regions of the Uttar Pradesh, India. They recorded minimum disease incidence in Machhra (20%) and maximum in Manikpur (62%).

Kadam *et al.* (2011) conducted field surveys in the Marathwada region of Maharashtra and recorded maximum disease incidence in Renapur Tahsil (17.8%) and minimum disease incidence in Nilanga (8.9%).

Windham *et al.* (2012) conducted a two year field survey to determine the effects of artificial inoculation techniques on the pathogenicity and virulence of *Aspergillus niger* kernel infection on two maize hybrids. *A. niger* kernel infection was significantly lower in the *A.*

flavus resistant hybrid compared to the *A. flavus* susceptible hybrid.

Venkatesh (2013) conducted survey during 2011-2012 around Coimbatore to assess the incidence of collar rot of peppermint. They recorded 93.66% collar rot incidence in Therkupalayam of Coimbatore District.

4.2 To study the Cultural, Morphological and Pathogenic Variability of pathogen.

Hayden *et al.* (1994) studied the biology of black mold (*A. niger*) on temperature and tropical onions and reported that more colonies of *A. niger* were persent on the leaves from tropical condition of Sudan as compared to temperature condition of U.K. *A. niger* is infrequent in soil and air but it is major component of onion bulbs grown in the Sudan because of high incidence in the field.

Araujo *et al.* (2004) evaluated different parameters influencing the germination of *Aspergillus* conidia. Inoculum concentration, age and temperature significantly influenced the germination.

Mohamed *et al.* (2013) isolated *Aspergillus* spp from different samples of infected peanut seeds and shells collected from different localities at Sharkia Egypt during 2008-2009 and 2009-2010. The isolates of *Aspergillus niger, A. flavus, A. flavus var. columnaris* and *A. parasiticus.*

Mohapatra *et al.* (2011) investigated pathogenicity of five isolates of *Aspergillus niger* collected from different agro-ecological situations. They recorded 60.33 to 75.00 per cent seed germination in artificially inoculated seeds , 90.66 in uninoculated control and 16.66 to 33.00 per cent seed rot.

Thomidis *et al.* (2012) observed the symptoms of post harvest rot on cherries collected from commercial orchards and causal agent was identified as *Aspergillus niger* based on cultural, morphological and pathogenicity tests. The lesions enlarged rapidly and separated easily from healthy tissue when pressure was applied and tissues were pale and water-soaked and the associated fungal spores were dark and powdery and easily liberated when mature.

Sharma *et al.* (2012) reported *Aspergillus niger* (black mold) on certain fruits and vegetables such as grapes, onions and peanuts. Microscopically, its conidiophores are smooth-walled, hyaline or turning dark towards the vesicle and conidial heads are bi seriate with the phialides borne on brown, often septate metulae. Conidia are globose, dark brown to black and rough-walled.

4.3 Management Stratiegies:-

4.3.1 Evaluation of Fungicides

Aulakh *et al.* (1974) were evaluated 8 fungicides in 2 seasons for control of collar rot (*Aspergillus niger*) and best fungicide was PCNB [quintozene] was found most effective followed by thiram.

Reddy *et al.* (1997) tested the efficacy of five fungicides against *A. niger, A. flavus, A. terreus* and *Rhizoctonia bataticola [Macrophomina phaseolina].* Among them carbendazim was most effective in inhibiting the growth *in*

vitro and carbendazim + thiram was found most effective in *in vivo* condition.

Ozer et al. (1998) evaluated the effectiveness of benomyl, thiram, prochloraz and tebuconazole against *A. niger* and *F. oxysporum in vivo* and *in vitro* conditions and found prochloraz (3 a.i./kg) significantly reduced the percent of post-emergence damping off.

Sheth *et al.* (2010) noted complete inhibition of mycelial growth of *Aspergillus niger* infecting citrus fruits by treating with carbendazim, Benomyl and carbendazim + mancozeb at 500 & 1000 ppm.

Vitavax power (carboxin 37.5%+ thiram 37.5%) was found highly effective against collar rot of groundnut by using as seed dresser (Shivpuri *et al.* 2011)

Mohapatra *et al.* (2012) recorded minimum collar rot incidence (4.66%) in seeds treated with Bavistin @ 2.0 g/kg of seed.

Rakholiya *el al.* (2012) observed minimum disease incidence (4.77%) and maximum pod yield (1632 kg/ha) with vitavax (4g/kg seed) followed by ipconazole.

Mohapatra *et al.* (2012) recorded minimum collar incidence (4.66%) at 30 days after sowing in seeds treated with Bavistin followed by Saaf (5.33), Thiram and Benlate (6.33%) over untreated control (62.00%).

Nandeesha *et al.* (2013) observed efficacy of systemic fungicides viz., carbendazim, propiconazole, tebuconazole and hexaconazole and non-systemic fungicides viz., mancozeb and captan against *A. niger* at 250, 500, 1000 and 1500 ppm concentrations. All systemic fungicides were found highly effective and completely inhibited the mycelial growth of the pathogen even at 250 ppm followed by mancozeb.

4.3.2. Evaluation of Phyto-extracts

Mohamed *et al.* (2012) observed that garlic cloves, onion and neem leaf extracts have the ability to cause

reduction in the mycelial growth of *A. niger* isolate from samples of infected peanut seeds.

Shakil *et al.* (2012) conducted a field experiment during 2009-2010 to evaluate the efficiency of aqueous extracts of five plant species, namely *Allium cepa*, *Allium sativum*, *Boerhaavia diffusa*, *Calotropis procera*, *Cannabis sativa*, and two fungicide Antracol and Ridomil against root rot of groundnut (*Arachis hypogaea* L.). Aqueous bulb extracts of *A. sativum* and *A. cepa* and the leaf extract of *C. procera* showed better disease management potential than that of the recommended doses of Antracol and Ridomil.

Sheth *et al.* (2010) observed the effectiveness of leaf extracts (10%) of tulsi *(Ocimum sanctum* L.), datura (*Datura stramonium* L:), lemon grass (*Cymbopogan flexuosus*), nilgiri (*Eucalyptus* sp.), citronella grass (*Cymbopogan winterians*), rhizome of ginger (*Zingiber officinale Rose*) and fruit extract of bitter gourd *(Momordica charantia* L.) against *A. niger* and found the ginger rhizome extract was found most effective in pre (8.50%) and post-inoculation (9.00%) treatments.

Premlata *et al.* (2012) evaluated the antibacterial and antifungal activity of crude extracts of fruit coat (Calyx) of *Withania somnifera* against *A. niger*. The extracts of *W. somnifera* also significantly inhibited the bacterial and fungal growth.

4.3.3 Evaluation of Bio-control agents and Organic Amendments:-

Rao *et al.* (2000) recorded least growth of *Aspergillus niger* in presence of *Trichoderma koningii* followed by *T. harzianum and T. hamatum in vitro* conditions. Resulted of pot experiments with *Trichoderma* spp used as seed+ soil treatment shown effective reduction in collar rot disease incidence.

Karthikeyan (1996) observed the effectiveness of organic amendments (neem cake, mahua cake, castor cake, farm yard manure, sheep manure and poultry manure), biocontrol agent (*T. harzianum)* and fungicides (carbendazim and thiram) to control seed and collar rot pathogen. The lowest disease incidence was recorded when

carbendazim was applied as a seed treatment whereas *T. harzianum* was found most effective as soil treatment.

Charitha *et al.* (2009) recorded the efficacy of *Trichoderma* spp. *and Pseudomonas fluorescens* (*in vitro*) against *A. niger. T. viride* was observed more effective in inhibiting the pathogen in pot culture. Combined effect of seed treatment with *T. viride* and captan significantly reduced the collar rot.

T. viride and captan was found significantly superior in reducing disease incidence when applied as seed treatment against collar rot pathogen (Devi *et al.*, 2009)

Mohapatra *el al.* (2011) recorded the antagonistic activity of four isolates (GV_1, GV_2, GV_3 and GV_5) of *Gliocladium virens,* one isolate each of *Trichoderma viride* and *T. harzianum* and a single isolate (NR7) of *Streptoverticillium* sp. The degree of inhibition was relatively more by GV_3 followed by $SVNR_7$ and GV_5

Gajera *et al.* (2011) observed antagonistic effect of 12 isolates of 3 *Trichoderma* strains (*T. virens, T. viride* and *T.*

harzianum) against the collar rot pathogen (*in vitro*) and observed that *T. viride* inhibited maximum (86.2%) growth of test fungus, followed by *T. harzianum* (80.4%).

Trichoderma viride treatment was found most effective in reducing disease incidence against *A. niger* causing collar rot in groundnut (Gangwar *et al.*, 2014)

Prabakaran *et al.* (2012) reported that *Bacillus* spp. was most potent against *A. niger* causing collar rot in groundnut.

Pratibha (2012) recorded the efficacy of *Trichoderma harzianum* (Th3) against collar rot disease of farmer field in the Jaipur district of Rajasthan.

Latha (2013) evaluated different fungal bacterial bio-agents against *A. niger* and found that maximum *in vitro* growth inhibition of *A. niger* of 65.6% and 62.2% was shown by *Pseudomonas fluorescens* (Pf1) and *Trichoderma viride* (Tv1), respectively, while among the chemical fungicides, Tebuconazole was the most effective. They also conducted field trials and reported least (20.45%) disease

incidence with bioformulation mixture of Pf1+Tv1+neem cake+FYM as against 60.0% in untreated control.

Nandeesha *et al.* (2013) evaluated the efficacy of 14 isolates of *Trichoderma* spp. In dual culture technique, the isolates TAG-2, TAG-13 and TAG-10 showed maximum mycelium inhibition percentage of (81.36, 78.51 and 75.97, respectively) against *A. niger.*

4.3.4 Source of Resistant

Aulakh *et al. (*1970) studied twenty groundnut varieties for resistant against *Aspergillus niger* under artificial conditions and only one exotic variety, U4-47-7 (EC 21115) from Sudan was found to be significantly resistant to the disease as compared with the other varieties.

Dasgupta *et al.* (1997) conducted in field screening trials during 1990-92 with selected groundnut germplasm lines against soil and seed isolates of *A. niger* in sandy soil.

They observed that lines C421 and C1780 were field resistant to both seed and soil isolates.

5. Research Methodology

5.1 Survey, Collection, Isolation, Purification and Pathogenicity of the Pathogen.

(i) Survey and Collection of Disease Samples

Survey will be conducted major groundnut growing districts of Rajasthan and diseased samples will be collected.

(ii) Isolation and Purification

Isolation from infected tissues of roots will be done under aseptic condition on Potato Dextrose Agar (PDA) medium and the culture will be purified with single spore or hyphal tip technique.

(iii) Pathogenicity test

Pathogenic nature of isolated isolates will be proved by seed and soil inoculation technique.

5.2 Variability

(a) Cultural and Morphological Variability

Cultural and morphological variability of different isolates will be tested on PDA.

(b) Pathogenic variability

Pathogenic variability in different isolates of *Aspergillus niger* will be tested in artificial conditions in cage house.

5.3. Management Strategies

Invitro study of fungitoxicity of different fungicides and plant extracts against *Aspergillus niger*

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Name and Signature of Supervisor

सह / सहायक आचार्य
सम्राट पृथ्वीराज चौहान
राजकीय महाविद्यालय, अजमेर

Suman Lora

Signature of candidate